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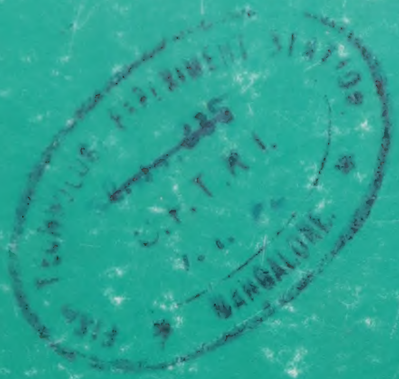
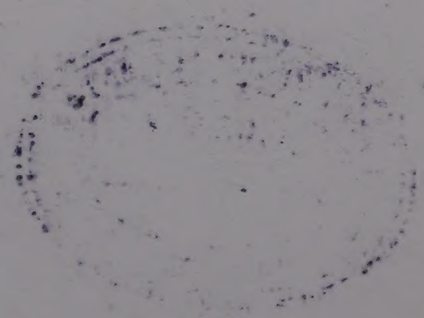


GUIDELINES FOR CHILLED FISH STORAGE EXPERIMENTS

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS



GUIDELINES FOR CHILLED FISH STORAGE EXPERIMENTS

by

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ABSTRACT

The paper contains comprehensive instructions for carrying out experiments to determine the chilled storage life of fish. These include methods for organoleptic evaluation of quality, determination of microbiological counts and estimation of chemical and physical parameters associated with quality. The purpose of the paper is to provide basic information and methods for institutes in developing countries as well as to promote a common approach in order to be able to compare results.

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INTRODUCTION

An essential prerequisite for designing the infrastructure for fish handling, storage, transport and marketing is to know how long each particular species in the catch will keep. Such information on the keeping qualities of most species from the colder waters of the northern hemisphere has been accumulated over many years by experience in the fish trade, and by scientific investigations carried out in technological institutes. Projections of demand indicate that more than 100 million tons of food fish per year will be required by the end of the century. To fulfil this demand will require that many species that are not exploited at present, or are converted to fish meal, must be brought into production for direct human consumption. Many of these will be from tropical or sub-tropical areas where scientific and practical knowledge is very limited. It is most urgent that this knowledge be acquired as rapidly as possible in order that rational development of an industry can take place. It is thus an opportune moment to consider what methods should be used for the conduct of storage experiments. Unfortunately, a good proportion of the work done previously is of limited value because of deficiencies in experimental design. Results are often conflicting, mainly as a result of poor control of raw material quality. This means that information obtained in one area cannot be reliably compared with that from elsewhere.

The following set of guidelines has been prepared in order to encourage technological institutes in developing countries to plan and carry out storage studies and to get the maximum value from them by being able to compare results. It may sometimes be considered that storage experiments carried out in a laboratory are of purely academic interest. This is not so, as a precise knowledge of the storage life and quality changes during storage, under good conditions, is necessary for anyone engaged in trade or inspection. Those examining fish, either in order to classify or purchase, need to know from an evaluation of the quality at the time of examination, how much storage life remains or, conversely, how much of the high quality storage life has been used up since catch.

The guidelines and methods given here concentrate on organoleptic assessment, backed up, if possible, by chemical and microbiological tests. Organoleptic evaluation is emphasized because, in the final analysis, acceptability is a function of appearance and eating characteristics. However, within the limits of available equipment and facilities, every attempt should be made to correlate organoleptic scores with a chemical or microbiological analysis.

There are many methods available, and within one method many variations between laboratories. The most important consideration is to use a standard method that is known to give reproducible results and not to change the method during the course of the investigations. The methods employed should always be specified when the work is written up. The methods given in this paper are not claimed to be superior in any way and are not set out in order to standardize methodology. They have been selected for people who may not have access to reference material. The basis of choice has been simplicity and proven reliability.

GENERAL CONSIDERATIONS

In any study to determine the keeping quality of chilled fresh fish it is necessary to have full control of the conditions in which the samples are collected, transported and stored, from the moment they are caught until the end of the experiment. The reliability of the results and the possibility of comparing them with results of similar trials elsewhere will depend very much on precise knowledge of the conditions throughout the experiment.

Another crucial point affecting the reliability of the experiment is the methodology employed to measure the changes in fish quality during storage. Sensory methods of assessment will play the most important role. It is questionable whether there is great value in carrying out non-sensory methods, since only a few of them have proved fully satisfactory for routine use particularly for tropical species. The selection of non-sensory methods, if any, should be based on the criteria of direct correlation with quality changes as assessed sensorily and the feasibility of carrying them out under the particular local conditions.

The interpretation and presentation of the results will also be very important. It is necessary to include in the description all factors that might have affected the estimated storage life so that comparison with similar studies will be possible.

WHICH SPECIES TO STUDY?

As a priority, storage trials should be concentrated on fish species that are already of commercial importance for the countries involved or those species whose estimated stocks and potential landing volumes are likely to have a similar or greater significance in the near future. A good example of the first group of species would be *Sardinella longiceps* in India, while *Opisthonema libertate* from Mexico is an example of the second.

WHICH CHILLING METHOD TO INVESTIGATE?

The characteristics of the local fishing fleet and the methods of fish preservation practiced on board, together with the possibilities for introducing changes should be taken into account for the selection of the storage trials to be performed. The nature of the utilization pattern after landing should also be considered. While icing fish and the use of CSW tanks can be studied on a reduced scale, it may be that RSW storage can only be studied on an industrial scale.

SAMPLE COLLECTION, TRANSPORT AND STORAGE

The collection of fish samples for storage studies is the most important step in experiments to establish the shelf life of a certain species in ice, CSW, or RSW. As the post-catch history of the fish samples should be precisely recorded, people carrying out the experiments should preferably collect their own samples. It is common to find in the literature storage studies using fish samples collected at fish markets or in the distribution chain. It is obvious that in these cases one can never be sure of all factors prevailing from the moment the fish were taken from the sea until they reached the market. This may be one of the main reasons why so many conflicting results are found in the literature.

Recognizing the difficulty of persuading people to work on board fishing vessels, the first decision must be who will collect the samples. A decision on what equipment is required must also be made.

During the collection of samples and their transport and storage, special attention should be given to recording all factors that could influence the course of the experiment by affecting fish quality and storage life, such as: catching operation, weather conditions, temperature of storage, etc.

During experiments with chilled fish, freezing must not occur at any time as it will significantly affect the results.

ICING

The type of ice which is used obviously depends on what is available but flake, tube, or crushed block ice is preferable to using whole blocks. If only block ice is available, then this must be crushed to a small particle size to prevent damage to the fish during transit.

Flake ice must be thin, so that when boxing the fish there will be good contact of fish while not causing indentations. The disadvantage of flake ice is that, due to its sub-zero production temperature (free flowing), and its larger contact area, it tends to aggregate more quickly into lumps, which can easily be broken but are a nuisance.

Tube ice is not as good for boxing as it slides off the fish, so that for good fish to ice contact, much ice is needed.

Crushed block ice is very sharp and irregularly sized, causing indentations and bad heat transfer. It should be crushed into pieces not bigger than 2.5 cm.

In practice a large variety of containers may be used to pack fish and ice for storage trials. However it is preferable to use plastic containers to guarantee cleanliness. Containers must have adequate drainage holes.

As a rough guide, the ratio of ice to fish is 1:1 but where possible more ice than fish should be used especially on long journeys. There should never be any air space at the top of the container, i.e., all spaces should be filled with ice. The ice is packed around the fish so that each fish is individually surrounded by ice. There should be a deep layer of ice at the bottom of the container, as this is the place where maximum melting of ice occurs.

CSW/RSW

Quick chilling of large quantities of small fish is only feasible in bulk if the fish are immersed in chilled sea water (CSW) or refrigerated sea water (RSW). CSW consists of a mixture of ice and sea water; air bubbling and/or water circulation provides mixing and quick heat transfer. The RSW system consists of a refrigeration unit that cools sea water held in a tank and takes away the extra heat load once the fish have been added to the tank.

A combination of RSW and CSW can be applied when extra cooling capacity is required, i.e., when fish is caught close to port so that there is not time enough for cooling the tank prior to fishing, or when the refrigeration capacity is limited.

Recent studies in FAO projects in Chile, Mexico and India on small pelagic fish have confirmed the technical and practical advantages of chilled sea water (CSW) systems over other methods, e.g., icing in bulk or in boxes and refrigerated sea water (RSW). However, there are some significant disadvantages and problem areas which must be investigated before the system can be widely introduced and one should be aware of these problems when carrying out a CSW storage trial. There have been reports of an "iceberg" effect due to compacted ice on trips of more than a few days, particularly in narrow containers. The "iceberg" prevents circulation and adequate chilling. The fluidization of small ice in the container with a limited quantity of water is said to prevent aggregation.

Mobile containers of different sizes are better suited to carry out trials with the CSW method.

QUALITY ASSESSMENT METHODS IN STORAGE TRIALS

Most research on fish spoilage has included chemical or microbiological assessment of fish quality. Although most of the tests used have serious defects, insofar as quality control techniques are concerned, they might be fully justified when it is necessary to have a more comprehensive knowledge of the spoilage pattern of a certain species. Of more immediate importance is the need to (i) estimate the storage life (keeping time) of these species when properly chilled, and (ii) to determine precisely the physical and organoleptic changes that occur during spoilage.

In order to achieve these goals, sensory methods are the most accurate and most widely used and therefore must be properly applied during these studies. Nevertheless, non-sensory methods can also be generally applied, but must be evaluated under local conditions.

Sensory Methods

A large number of schemes have been proposed for sensory evaluation and many are currently in use in various institutes and industries. In some cases, in industry, it is sufficient to have a pass/fail system that determines whether fish is fresh enough for processing or marketing. However, when carrying out storage trials it is necessary to make a precise estimate of the quality and to assign a value to it. The measurement of freshness

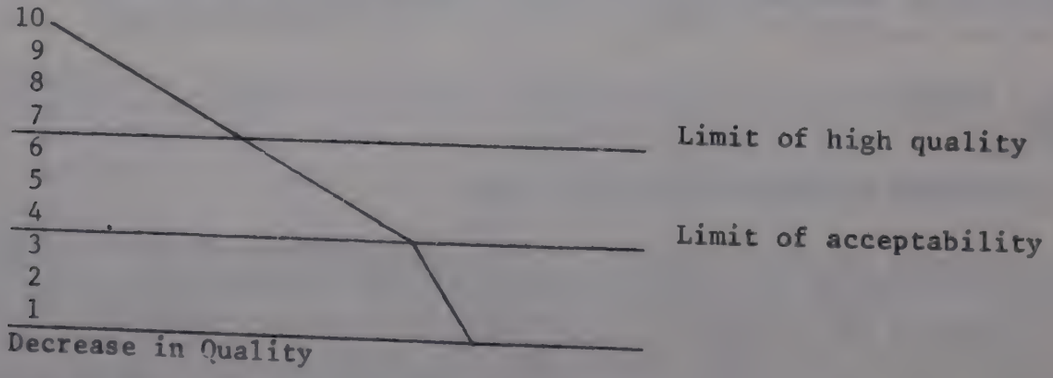
in a quantitative manner is a difficult concept to explain. Most people tend to think in terms of preference or like/dislike when asked to taste fish, but such value judgement or hedonic scales are only of use to measure likely consumer response to a product. Of course, tests like this, triangle tests, pair comparisons, etc., have their place in the product development laboratory but for quantitative evaluation of quality a more objective system is required. The scale used assumes that the quality ranges from absolutely fresh (just caught) to absolutely putrid. Freshness can be related to quality along a continuous line between the opposite ends of the scale. It is most usual to use a 10-point scale from 1 to 10 in which for the purposes of statistical analysis the intervals between each score mark are assumed to represent equal differences in quality

Table 1

High quality	Excellent	10
	Extremely good	9
	Very good	8
Limit of high quality	Good	7
Acceptable	Acceptable	6
		5
Limit of acceptability	Fair	4
Reject	Poor	3
	Very poor	2
	Extremely poor	1

In practice the correlation between score mark and quality tends to curve at the lower end. If one sets a score mark of 4 as the limit of acceptability then the tasters tend not to score products which they would reject and thus make less use of the lower end of the scale.

Figure 1



DISTORTION OF THE CURVE OF QUALITY DECREASE VS. SCORE

By training, tasters can learn to distinguish smaller differences in freshness and place samples more accurately on a freshness continuum between completely fresh and putrid. This position can then be transferred to a quality scale and a score mark assigned. A freshness scale can be based on descriptions of quality, and workers should attempt to build up a descriptive profile of each species during storage experiments. In order to describe samples more precisely it is valuable to include other questions when submitting them to tasters. A questionnaire that has been used successfully is set out below, although many variations are possible and modifications may be required for some species.

There is, therefore, a basic need for any institute of fish technology involved in storage trials to develop and maintain an experienced taste panel. The organization and training of taste panels cannot be discussed here but they are well covered in many papers and text books available in food technology libraries.

Table 2

MODEL OF QUESTIONNAIRE

CODE				
Colour - normal				
	discoloration			
Odour/flavour				
	fresh and characte-ristics of species			
	loss of odour/ flavour			
	neutral			
	off-odour/flavour			
	non-specific			
	rancid			
	old/stale			
	spoiled			
Texture	firm			
	soft			
	tough/stringy			
Other comments				
Overall quality score				
_____ Name		_____ Date		

After all the preliminary steps have been taken to organize and train a group of people for sensory assessment of fish quality it is advisable to point out the convenience of practical training with local species. In fact, an essential preliminary task is to store batches of local fresh fish and try to describe day-to-day changes of physical and organoleptic characteristics. These should be considered in group discussions to try to reach a common opinion on good or bad characteristics.

Although taste panels should consist of at least six trained members, it is recognized that in some laboratories this may not be possible. Shortage of people should not preclude

carrying out tasting tests but the results will not be as amenable to rigorous statistical interpretation. The scheme given in Table 3 may prove useful for training tasters. Beginners can start at the left side of the table with only two classifications, Accept/Reject. With more experience they can move toward the right, first with three classifications and finally with the full 10-point scale.

Table 3

CLASSIFICATION AND SCORING SYSTEM FOR FRESHNESS BASED ON ODOUR AND FLAVOUR OF RAW AND COOKED FISH

		Score		Score
Acceptable	No off-odour/flavour	I	Odour/flavour characteristics of species and very fresh, seaweedy loss of odour/flavour neutral	10 9 8 7 6
	Slight off-odour/flavour	II	Slight off odours/flavours such as mousy, garlic, bready, sour, fruity, rancid	5 4
	Limit of acceptability			
Reject	Severe off-odour/flavour	Reject	Strong off-odours/flavours such as stale cabbage, NH_3 H_2S or sulphides	3 2 1

Non-Sensory Methods

Chemical Methods

A variety of chemical compounds or groups of compounds accumulate post mortem in fish flesh. These chemical compounds are intermediates or end products of biochemical changes occurring in the muscle of fish after they have died or result from the action of exogenous bacterial enzymes released by the proliferating bacteria on muscle metabolites. The amounts formed can be used as an index of spoilage. However, post mortem changes vary considerably between species and within the species, according to fishing ground and season. No chemical test, therefore, can be generally applied, but must be evaluated under local conditions.

Comments will be presented here on the advantages and disadvantages of the main chemical methods which have been used as an index of spoilage for fish. Taking into account the specific storage trial involved, national institutes should decide which of them they will employ, if any.

Proximate analysis

Because of the influence of chemical composition on keeping quality, it is recommended to determine the proximate chemical composition (moisture, fat, protein, ash) of the fish samples under investigation. This determination need only be done on the first day of sampling in a storage trial. However, since chemical composition varies with season and catching ground, one must also make determinations in different seasons and in various areas.

Total volatile bases (TVB)

Most of the component called total volatile base (TVB) consists of ammonia (NH_3) and trimethyamine (TMA) and reflects the action of spoilage organisms on trimethyamine oxide (TMAO). In some fish species there is a good correlation between TVB or TMA and sensory assessment of freshness. However, one serious defect is that volatile bases mainly accumulate in the fish flesh during the later phase of spoilage after the bacterial population has grown. For most fish species the TVB content in the flesh is low during the edible storage period and only when the fish is near rejection level increasing amounts of TMA and TVB are found. Despite this, estimation of TVB and TMA are the objective methods most widely used in fish quality assessment.

It is not possible to give a precise figure of TVB level for acceptance/rejection as this varies from species to species and to a certain extent between consumers. However, rejection levels are normally in the range of 20-30 mg of TVB nitrogen per 100 g of muscle.

Total volatile acids (TVA)

The test for these compounds (formic, acetic, propionic and butyric acid) volatile or steam-volatile acids, suffers the same handicap as the assessment of TVB as TVA only accumulate in the fish flesh during the later phases of spoilage. However, the method may be useful for some fish species.

Volatile-reducing substances (VRS)

Measurement of VRS attempts to evaluate the total odoriferous substances present in a sample. These strongly smelling substances such as lactic acid, hydrogen sulphide, amines, mercaptans, indole, etc., are formed in spoiling fish as a result of bacterial action in the later phases of spoilage. The test is therefore also of limited use.

Hypoxanthine (Hx)

This substance accumulates in the fish flesh as a result of nucleotide degradation. ATP is degraded via inosine to Hx mainly due to autolytic processes, but in the later phases bacterial action may also be involved. Measurements of Hx should therefore give a good indication of early post mortem changes in fish. However, great species differences in the speed of nucleotide degradation have been demonstrated, and this is a major factor in limiting the usefulness of the Hx test.

The advantage of using Hx as an indicator of freshness is that there is no lag phase in its formation and that it reflects autolytic as well as bacteriological deterioration. Unfortunately, the determination of hypoxanthine is rather complicated, requiring the employment of ion exchange resins and a spectophotometer. Because of the complexity the method is not given in this paper and can be found in many other publications.

Peroxides

The often highly labile fat in fish is susceptible to oxidation. In the early phases peroxides are formed, and these compounds, being odour and flavour-less, can often be detected chemically before any rancidity becomes apparent. The peroxides are eventually further oxidized to aldehydes and ketones, which have a very disagreeable "fishy" or "rancid" odour and taste. However, depending on the fish species and storage conditions a good correlation between peroxide value and organoleptic quality may be found. In fact, estimation of the fat peroxides has been the most widely used chemical test for rancidity.

Others

There are a number of other tests designed for measuring the chemical changes occurring in fish during chilled storage. Mention should be made of the determination of hydrogen sulphide (H_2S), histamine and TBA.

When fish spoil under anaerobic conditions (RSW storage without aeration, or when in close contact with dirty surfaces) but also during spoilage of ungutted fish, H_2S is found in varying amounts. Therefore, in the case of small pelagic fish which is always stored ungutted and most commonly in RSW, the determination of H_2S could be an important test to perform.

Histamine is formed in the fish flesh as a result of bacterial decarboxylation of histidine. Since some fish have a high initial content of histidine, large amounts of histamine may be formed before any spoilage is apparent. Thus histamine content is not a good indication of organoleptic quality but, being toxic to man, its determination is an important safety aspect.

The 2-thiobarbituric Acid (TBA) method was found to be effective for the determination of oxidative rancidity in a wide variety of fish and fishery products. The method involves distillation of malonaldehyde from an acidified homogenate to form a coloured complex with TBA that can be measured by spectrophotometer. Unfortunately, the method depends on precise control of heating rate, distillation time, etc., and so is not readily reproducible between laboratories. It can, however, be used as a comparative method to follow storage changes.

Physical methods

pH

The early post mortem changes in fish are associated with a drop in pH from 7.07-7.2 to 6.2-6.5 due to the predominant formation of acid compounds. The pH in the muscle increases again during the later phases of spoilage, when volatile amines and other basic compounds predominate. However, the pH changes depend on a variety of factors such as fish species, fishing ground, catching method, feeding of the fish, etc., which make pH determination a very unreliable index of freshness. Additionally, a spot measurement does not indicate whether it is taken during the decreasing or the increasing part of the curve.

Torry Fish-Meter

Based on the principle that electrical resistance in tissues decreases progressively after the death of fish, some instruments have been designed for the rapid measurement of the degree of freshness of wet fish. The most recently developed instrument is the GR/TORRY Electronic Fish Freshness Meter. The method is considered useful, but cannot replace organoleptic judgement. The readings are sensitive to physical damage and the temperature of the fish. Separate calibrations are necessary for different fish species. Great fish-to-fish and even apparatus-to-apparatus variations are seen, but further work on improving the device may eliminate some of the drawbacks.

Microbiological methods

It is generally accepted that bacterial growth predominates over other causes of deterioration of fish and in order to get a picture of the bacterial activity on the fish the easiest and most practical method is to determine the total bacterial count.

It is often the practice to carry out total bacterial counts at temperatures near 35°-37°C although it has long been known that the microfloras responsible for putrefaction of fish are psychophiles. Therefore, counts at that incubation temperature have no correlation with organoleptic quality. Incubation temperatures of 0°-4°C and 20°-25°C are recommended for the determination of fish spoilage bacteria. Results obtained with these temperatures would give only slight differences on the qualitative side. However, taking into account the longer incubation period needed at 0°-4°C (20-25 days), it is preferable to employ incubation temperatures of 20°-25°C.

As *Pseudomonas* form the main group of bacteria responsible for chilled fish spoilage, counts of this genus utilizing special culture media can offer a better correlation with the organoleptic quality.

Fish samples for bacteriological analysis may be collected from different parts of the fish (skin, flesh, guts, gills) and several collection methods are used for this purpose (cut, swab, excise, press). These methodological differences have contributed very much to controversial results shown in the literature and very often do not allow direct comparisons. According to the evidence supporting the surface concept in measuring fish spoilage it is by all means recommended to take fish skin samples. The aseptic excision method is the superior technique to take off these fish skin samples. However, taking into account the size of most small pelagic fish and the need to standardize this important methodological step in order to obtain consistent comparable results, a different approach was developed by Danish researchers for these species. Using a pair of scissors they cut a transverse section of 10 g from the part immediately anterior to the tail insertion of the fish. The fish sample consists of skin, flesh and bones. The method is simple, quick and avoids foreign contamination. Care should be taken in order not to include any viscera in the sample, which could easily be the case with a very small specimen.

INTERPRETATION OF RESULTS

It is extremely difficult and hazardous to state absolute values for the storage life of chilled fish, since it varies very much with methods of catching, handling, size, seasons, fishing grounds, etc., and depends largely on the fat content and the degree to which fat content is a problem. Therefore, caution should be taken to all these factors when interpreting specific results.

For some purposes it is sufficient just to establish how long fish will be fresh enough for human consumption but there are other instances where a more precise and accurate estimate must be made. This is so when a product is to be made to a defined and consistent standard, e.g., canned sardines.

Freshness of fish can be measured satisfactorily by sensory techniques, but both the precision and, to some extent, the accuracy of the method must be established. The performance of the panel as a whole and of individual members can be monitored by statistical methods. The statistical analysis is quite straight-forward and, now that sophisticated desk-top calculators are widely available, should be routinely carried out.

PRESENTATION OF RESULTS

In presenting results of storage trials it is necessary to characterize all factors related to the fish samples investigated. Intrinsic and extrinsic factors must be defined. Among the intrinsic factors one should present data on taxonomy, size, weight, shape, fat content, biology and food habits. Concerning the extrinsic factors a concise description should be given of the catching ground, fishing operation, temperature of sea water, air and fish, handling and chilling procedures, transport and storage conditions.

Sampling and analytical procedures should be well defined generally by quoting the reference method. If any change is introduced to the method or if a new method is used this should be fully explained and described.

Results must be presented in a simple, objective and understandable way, commonly by utilizing tables and graphs. Very special care should be given to the description of the organoleptic changes during the storage trial in order to offer the most precise picture of these changes.

The discussion of the results should include all factors that could have affected the experiment and particularly the estimated storage life. Discussion should also cover any possible correlation between the organoleptic changes and the physical, chemical and bacteriological parameters. Finally, the practical implications of the results should be fully discussed, mainly in the light of local conditions of fish handling and utilization.

SUGGESTED CHECKLIST AND STANDARD PROCEDURES

PLANNING

Plan your experiment carefully and with enough anticipation

Review specific literature on the subject

Make proper arrangements for collection of samples, such as by identifying which fishing boat will be used, which person will go on board, which equipment and utensils should be taken on board and how they will be put there

Make proper arrangements for the transport of samples from the landing place to the laboratory

Make proper arrangements for the storage of samples

Have your taste panel organized and prepared

Have your laboratory material and personnel organized and prepared

COLLECTION OF FISH SAMPLES

Once fish is taken from the sea, transfer them as quickly as possible to the chilling media

Avoid all possible heavy contamination during the handling operation

Try to have your fish sample as uniform as possible regarding size, weight, and catching time

Do not mix different fish species in the same box, container or tank

ICING

Use as a rough guide a ratio of ice to fish of 1:1. Where possible use more ice

Ice should be packed around the fish so that each fish is individually surrounded by ice

There should be a deep layer of ice at the bottom of the box

The top of the box should also receive a deep layer of ice in order that all spaces are filled with ice

Protect filled boxes from sunlight, rain and wind

CSW

An insulated container of at least 100 l capacity should be used

The proportion of ice, seawater and fish as recommended for commercial practice is seen in Table 4

If a small container is used (about 100 l), an extra amount of ice should be utilized (10 kg)

The ice is put in the container on land

Just before fish is added the proper amount of seawater should be introduced and mixed with the ice

Add the fish while thoroughly mixing the ice and seawater slush

An extra amount of ice should then be put on top

Measure fish temperatures at different points in the container or tank hold, every 15 min during the first hour and then once per hour

Table 4

PROPORTIONS OF ICE, SEA WATER AND FISH IN CHILLED SEA WATER HANDLING (CSW) OF SMALL FISH

Sea water temp. °C	crushed ice						tube ice						flake ice					
	ice		water		fish		ice		water		fish		ice		water		fish	
	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%
15	127	14	73	8	720	78	127	14	73	8	720	78	127	14	73	8	720	78
20	161	17	42	5	718	78	162	18	63	7	698	75	163	18	84	9	678	73
25	193	21	48	5	684	74	195	21	73	8	660	71	197	21	94	10	630	69
30	224	24	52	6	652	70	226	24	79	9	626	67	228	24	102	11	604	65
35	252	27	56	6	623	67	255	27	84	9	596	64	257	27	108	12	572	61

The proportions shown in the above table will provide adequate cooling capacity to 0°C, while keeping fish carrying capacity at a maximum.

Meltage during transportation of ice or as a result of bad insulation of the hold or container is not taken into consideration.

Differences between types of ice are a result of different bulk densities of the ice; the more void the more water is needed to make up a slush.

RSW

The temperature of the sea water in the tank should be close to 0°C before the fish is added.

Just before landing fish is taken out by brailing and put into an insulated container and topped up with sea water from the RSW tank which should be around 0°C.

RECORDING

Make a record of all factors that could influence the development of the experiment, affecting fish quality and storage life:

Catching operation

Weather conditions (sun, rain, wind)

Temperature of sea water, air, fish

Handling operation

Period of time lapsed from taking fish from the sea water and transferring it to the cooling media.

Full description of the container used or fish hold:

Container - dimensions, lining, insulation, cleanliness, shape

Fish hold - capacity, shape, inner lining, insulation, circulation and mixing (for CSW/RSW), refrigeration capacity, refrigeration

Ice - type, quantity, transport, loading time, observations after sea water addition (caking, slush, etc.) for CSW trials

Sea water - quantity, temperature, loading time

Fish - species, quantity, size/weight distribution, loading time, temperature

Mixing (CSW) - Were ice, sea water and fish mixed well? Were all fish under water? How long did air blower operate?

Read the circulating water temperature before and after the refrigeration units of RSW systems

Full description of the unloading procedure

Full description of the transportation procedure from the landing place to the storage site

STORAGE

During experiments with chilled fish, freezing must not occur at any time as it will significantly interfere with the experimental results

With iced fish, ice should be "topped up" daily, including weekends

With iced fish, in boxes, the containers should allow drainage of melted water

In the case of CSW or RSW storage trials the container with the fish samples and sea water should be kept preferably in a chill store at around 0° to 1°C. Frequent checks should be made on temperature

Again it is necessary to make a record of all factors relevant to the storage conditions which could affect the experiment:

Full description of the storage place,

Ambient temperature and its variation during storage

QUALITY ASSESSMENT

Sampling

The trials are usually of 2-3 weeks' duration and samples should be taken, if possible, every 2 days, i.e., twice during a working week. This usually involves work at weekends to complete some of the analyses.

Take 6 fish for each sampling day. If fish are particularly small, more fish must be used

All fish used during the sampling should be kept on ice

The fish should be held by the head and handled as little as possible

If available, protective disposable gloves should be worn during microbiological sampling

The six-fish samples are weighed

Samples are taken for bacteriological analysis

If available, GR Torry Meter readings are taken

Visual and olfactory assessment is then carried out on all six raw fish

The six fish are then filleted, unless they are too small

Six fillets (being 3 of one side and 3 of the opposite side) are quickly well washed under running water and will be used by the taste panel

The last six fillets are not washed and are used for the chemical analysis

(Care should be taken not to contaminate those parts of the fish to be used for microbiological analysis)

PROCEDURE FOR ANALYSIS

GR Torry Meter

Three readings are taken on the left hand side of each fish

The mean reading is calculated for each fish and then for the whole sample. This will indicate the range and the mean value obtained for each sampling day

ORGANOLEPTIC ASSESSMENT

Raw Fish Examination

Each fish is examined to assess the condition of the eyes, gills, body cavity, flesh and skin

The examination is in general only performed by the experienced technical staff who are responsible for the storage experiment

The examiner should record the following characteristics for each fish:

Pigmentation of the skin and its mucus

Eye tint and shape

Gill tint and odour

Rigidity and colour of the flesh and the abdominal wall

Cooked Fish Examination

Preparation of samples

Generally one fillet is used from each fish sample. In the case of very small fish the whole fish should be used, after heading and gutting. From bigger fish, part of the fillets or a cross section (a steak) is used. In all cases the sample should include muscle tissue from the belly flap as well as from the back of the fish.

Undesirable membranes are removed and the samples should be well and quickly rinsed under running water. If possible, bones should be taken out.

The fillets should preferably be individually placed in nylon bags which are sealed, immersed in boiling water and cooked for 20 min. If nylon bags are not available the fillets may be steamed individually in a steamer or a beaker, covered with aluminium foil, or partly submerged in boiling water inside a covered pan, for 15 min. No water or condiments are added. Having selected one method of cooking it is important to keep to it.

Serving

Use a panel of at least 6 people. Each member will receive a different fish to taste.

Normally, it is recommended to place in front of each panelist the following: (1) questionnaire, (2) fork, (3) napkin, (4) glass of water, (5) a piece of tasteless bread, and (6) a pouch or disposable cup for spitting in.

After the heat treatment, the sample is placed on a white plate which has been coded with a letter/figure combination. The samples are served to the panelists in such a way that the order of the samples from left to right is different from panelist to panelist.

Assessment

The panelist smells the different parts of the sample. In case of a sample with skin on, the smell underneath the skin should be taken into account. Then the sample is tasted. The panelists are asked to taste different parts of the sample. If more than one sample is being assessed there should be an interval between tastings. The mouth can be cleansed by eating bread and drinking water between the tastings.

The judgement of the sample is reported both by a score mark and by checking the specified characteristics listed in the questionnaire.

During judging the room should be absolutely quiet.

After tasting every panelist should tidy up his place and deliver the questionnaire to the person in charge of the tasting.

BACTERIOLOGICAL EXAMINATION

Sampling

The skin sample is taken by excision from each of six fish of a piece of skin with an approximate area of 10 cm^2, with the help of sterile tools (template, scalpels and forceps). One should try to excise the skin sample with the minimum possible amount of flesh attached to it.

In the case of small pelagic fish, aseptically cut, with a pair of scissors, a transverse section of 10 g is taken from the part immediately anterior to the tail insertion of each fish. Care should be taken in order not to include any viscera in the sample. If fish is too small, use more than one fish to obtain a 10 g sample.

Preparation of the homogenate and of the decimal dilutions

Each one of the 6 samples should be individually treated.

Place the sample in a stomacher bag. Add 100 ml of peptone water and mix the contents in a stomacher for 5 min. If a stomacher is not available each sample is placed in a sterilized 150 ml conical flask containing 10 glass beads and then shaken vigorously in 100 ml of peptone water for 5 min. Alternatively, a blender with a sterile jar can be used.

From this initial homogenate, a tenfold dilution series is prepared using 9 ml quantities of peptone water. Each successive dilution will decrease the concentration tenfold.

Pour plating

Plate count agar plates are innoculated in duplicate by pour plating 1 ml of the appropriate dilutions. A set of plates containing Masurovsky's medium is also used to determine the level of pseudomonads.

Incubation

Invert the prepared duplicate set of plates of each type of agar and incubate them at 20°-25°C for 4 days.

Counting the results

Examine the plates after the prescribed incubation period. If this is not possible, they may be held at 4°C for a maximum of 24 h.

Count the colonies in each plate containing between 30 and 300 colonies.

Expression of results

Give the result as the number of aerobic bacteria per cm^2 of fish skin or g of fish. Express it by a number in the range 1.0 to 9.9 multiplied by 10^x, x being the appropriate power of 10.

Method of calculation

When counting several situations may be encountered:

(i) If the plates examined contain no colonies, give the result as less than 1×10^1 bacteria per g or cm^2, 10^1 being the inverse of the dilution of the fish homogenate (example 1)

(ii) If the plates corresponding to the fish homogenate (1 in 10) contain less than 30 colonies, give the result as less than 3×10^2 bacteria per g or cm^2 (example 2)

(iii) Generally, however, there is at least one plate which contains between 30 and 300 colonies. In this case, count all plates corresponding to the dilution or to the 2 successive dilutions in which this plate or these plates are located. For each dilution calculate the average number of colonies. Retain only 2 significant digits. Thus, for a 3-digit number, round off to the nearest zero. If the third digit is 5, round off to the lower zero. Multiply the value obtained by the inverse of the corresponding dilution to obtain the number of bacteria per g or cm^2. In a case in which there are 2 values for the number of bacteria per g or cm^2 (as when 2 dilutions have been retained) average these 2 values if the ratio of the higher value to the lower value is less than 2. If not, retain the lower value (examples 3, 4 and 5).

Examples	No. of colonies from 1 g of food homogenate (1 in 10)	Results (in No. of bacteria per g of product)	Explanations of Calculations
No. 1	0	fewer than 1×10 bacteria	$1 \times 10^1 = 1 \times 10$
No. 2	18 17	fewer than 3×10^2 bacteria	$30 \times 10^1 = 3 \times 10^2$

Examples	Number of Colonies dilution at $\frac{1}{100}$	Number of Colonies dilution at $\frac{1}{1\,000}$	Ratio	Results (in No. of bacteria per g of product)	Explanations of Calculations
No. 3	175 208	16 17	-	1.9×10^4	175 + 208 = 383 ÷ 2 = 191 → 190 → $190 \times 10^2 = 1.9 \times 10^4$
No. 4	322 278	23 29	-	3×10^4	322 + 278 = 600 ÷ 2 = 300 → $300 \times 10^2 = 3 \times 10^4$
No. 5	296 378	40 24	<2	3.3×10^4	296 + 378 = 674 ÷ 2 = 337 → 340 → $340 \times 10^2 = 3.4 \times 10^4$ 40 + 24 = 64 ÷ 2 = 32 → $32 \times 10^3 = 3.2 \times 10^4$ $\frac{3.4 \times 10^4}{3.2 \times 10^4} < 2$ $\rightarrow 10^4 \frac{(3.4 \times 3.2)}{2} = 3.3 \times 10^4$
No. 6	327 330	18 25	<2	2.7×10^4	327 + 330 = 657 ÷ 2 = 328 → 330 → $330 \times 10^2 = 3.3 \times 10^4$ 18 + 25 = 43 ÷ 2 = 21.5 → 21 x $10^3 = 2.1 \times 10^4$ $\frac{3.3 \times 10^4}{2.1 \times 10^4} < 2$ $\rightarrow 10^4 \frac{(3.3 + 2.1)}{2} = 2.7 \times 10^4$
No. 7	spreaders spreaders	18 24			18 + 24 = 42 ÷ 2 = 21 → $21 \times 10^3 = 2.1 \times 10^4$

(iv) In some special cases, there are no plates containing 30 and 300 colonies. If the number of colonies differ slightly from these limits at the level of 2 successive dilutions, proceed as for iii (case for 2 retained dilutions (example 6). If the plates corresponding to 1 dilution contain spreading colonies, and if the number of colonies of the next dilution is lower than 30 proceed with this dilution also as for iii (example 7).

CHEMICAL AND PHYSICAL EXAMINATION

pH

Duplicate 2 g samples are homogenized with 10 ml of neutralized 0.005 M sodium iodoacetate (pH 7). The pH is measured at room temperature using a pH meter with a glass electrode.

Sodium iodoacetate is poisonous. Do not pipette by mouth

Check pH meter calibration before taking readings

Thoroughly clean pH electrodes between readings

If sodium iodoacetate is not available pH can be determined in a distilled water homogenate if measurement is not delayed

Preparation of sample for proximate composition and chemical analysis

The six fillets (one from each fish) are chopped finely with a knife or scalpel and pooled. Skin and large pieces of connective tissue should be removed.

Determination of dry matter or moisture

Apparatus: Small porcelain dishes or covered aluminium moisture cans
Balance 0.0001 g
Oven 105°C

Place dish or moisture can in an oven at 101°-105°C for two hours, then cool in a desiccator for 20 minutes. Weigh, add 2-10 g of sample and weigh again. Put in oven for 20-24 h or overnight, cool in desiccator for 20 minutes then weigh again. (In order to reduce washing-up, small circles of aluminium foil can be pre-cut to fit moisture cans and subsequently thrown away together with the sample, if this is not required for ashing.)

Calculation

$$\frac{\text{Wt. of dish with dried sample - wt. of dish}}{\text{Wt. of dish with "wet" sample - wt. of dish}} \times 100 = \% \text{ dry matter}$$

Ash content

Apparatus: Same as for dry matter plus a muffle furnace operating at 450°C.

The porcelain crucible together with the dry matter sample can be ashed or the process can start again from weighing the wet sample into the crucible. In both cases the contents must be charred in the crucible (Bunsen burner, while holding the crucible on a pipe clay triangle or a tripod). After charring, ash in the muffle furnace 450°-650°C until constant weight is achieved after cooling in a desiccator (usually more than 5 h).

Determination of oil content and peroxide value

Apparatus: (including special glass apparatus)

Meat chopper
Drying cabinet
Desiccator

Homogenizer, e.g., MSE-mixer
Centrifuge with 100 ml tubes
500 ml conical-shaped flasks with wide mouths and rubber stoppers with 2 tube fittings, about 1 cm in diameter,

Or

500 ml ground neck flasks with specially made stoppers with 2 tube fittings and glass tap

Reagents

Chloroform
Acetic acid
Potassium iodide, saturated solution freshly made (13 g in 10 ml water)
Starch 1.0 g is dissolved in 100 ml boiling water, cooled, and filtered
A little mercury iodide is added
1/500 sodium thiosulphate, freshly made
Deoxidized Co_2. CO_2-cylinders may be emptied of possible content of oxygen by opening the valve slightly at the top for a couple of days before using the cylinder

Determination of oil content by the method of Bligh and Dyer

A quantity of 15 g of muscle is weighed out and transferred to a blender. Water must be added to some samples depending on the water content to ensure that the final ratio, chloroform:methanol:water is 1:1:0.8.

Chloroform (15 ml) and methanol (30 ml) are added, and blended for two minutes. Then an additional 15 ml chloroform is added and the material is blended for half a minute. Finally, 15 ml water is added and the sample is blended again for half a minute.

The contents of the blender are transferred to a centrifuge tube and centrifuged for 15 minutes at 3 000 rpm. Counterbalancing may be avoided if the tubes have almost the same weight when empty. After centrifuging, the water phase at the top of the tube is removed. The material to be analysed is loosened with a spatula and the chloroform phase is poured through a filter, moistened with chloroform, into a small flask, which is then stoppered.

Determination of oil content

A 5-10 ml sample of extract is placed in a small beaker which has been dried and weighed. The chloroform is evaporated and the sample is dried in an oven for one hour at 105°C, cooled in a desiccator and weighed.

Determination of peroxide values

A 500-ml conical-shaped flask equipped with a rubber or glass stopper and tube fittings is aired with CO_2. The airing continues during the addition of reagents. Ten-ml extract, 15 ml acetic acid and 1.2 ml saturated potassium iodide are added, whereafter the flask is closed with a stopper and allowed to stand in a dark place for one hour.

Then 30-50 ml water and 1 ml starch solution are added and then flask titrated with 1/500-N thiosulphate until the blue colour disappears.

The result is given in milli-equivalents peroxide/1 000 g oil as follows:

$$\frac{\text{ml 1/500-N thiosulphate} \times 2}{\text{g oil in 10 ml extract}}$$

Determination of protein in fish muscle

Protein is determined by estimating the quantity of nitrogen and multiplying by a factor of 6.25 to convert to protein. There are a number of variations of the Kjeldahl method, one of which is given here.

Apparatus

Hoskin's Kjeldahl apparatus
Digestion heaters
Flasks Micro Kjeldahl (30 ml capacity)

Reagents

Digestion Mixture

32 g K_2SO_4	(AR)	well mixed - allow to cool before use
5 g $HgSO_4$	(AR)	
1.4 g SeO_2	(AR)	
100 ml H_2SO_4	(SG 1.84) (AR)	

Sodium hydroxide/Thiosulphate mixture

Aqueous solution of 40 g NaOH (AR) + 5 g $Na_2S_2O_3$ (AR)/100 ml H_2O

Standard N/70 H_2SO_4 or HCl

Standard Borate Solution (1 percent boric acid)

To make one litre of reagent - 10 g pure boric acid is introduced into a 1 litre flask, 200 ml ethyl alcohol is added, then about 700 ml distilled H_2O. Boric acid is brought into solution by shaking and 10 ml mixed indicator added. After mixing the contents are brought to the desired end-point of faint reddish; this usually requires the addition of a little alkali. The mixture is then made up to the mark.

Mixed Indicator

This contains bromocresol green 0.033 percent and methyl red 0.066 percent in alcohol. It keeps indefinitely.

Burette

Graduated in 0.01 ml division.

Procedure

A sample of fish muscle 0.1→0.25 g is introduced into a Kjeldahl flask, 5 ml of digestion mixture added, gently heated until all charring ceases - then strongly heated until digestion is complete (i.e., colourless) approximately 1.0-1.5 h - cool. Contents of flask washed into standard volumetric flask with distilled H_2O - make up to volume (i.e., say 100 ml - according to quantity of NH_3-N present in initial sample).

Take aliquot portion (i.e., 10 ml) - introduce into Hoskin's distillation apparatus - add sufficient NaOH - $Na_2S_2O_3$ solution to neutralize - then stem distil into standard borate solution (10 ml) making sure condenser dips beneath the borate solution - continue distillation for 7-10 minutes after first drop of distillate has left condenser. After distillation remove flask, washing down, etc., with distilled H_2O - titrate contents of flask to faint red with N/70 H_2SO_4 and correct for titration by completing necessary blank determination on reagents and borate solution.

1 ml N/70 HCl or H_2SO_4 = 0.2 mg N

N x 6.25 = protein

Total volatile bases (TVB)

Apparatus

Conway dishes, glass plates
250 ml beakers
1 and 2 ml pipettes
Funnels, filter paper
Incubator (37° C)
Microburette (5 ml)
Conical flasks (150-250 ml)
Homogenizer

Reagents

2 percent boric acid
methyl red
saturated potassium hydroxide
phenolphthalein
0.05 M (0.1 N) sulphuric acid

Procedure

Prepare an extract by homogenizing 50 g of fish muscle in 100 ml of 2 percent boric acid solution. Weigh accurately to 4 decimal places.

Place 2.0 ml of the boric acid extract in the outer chamber of the Conway dish. Prepare samples in triplicate.

Place 2.0 ml of 2 percent boric acid in the centre chamber of the Conway dish, and add a drop of screened methyl red indicator.

Place the greased ground-glass plate over the unit so that the central chamber is closed, but the outer chamber open.

Add 1 ml of saturated potassium hydroxide (CARE - HIGHLY CAUSTIC - DO NOT PIPETTE BY MOUTH) to the outer chamber, and add a few spots of phenolphthalein indicator. Move the lid carefully to close off the whole of the unit.

Mix the contents of the outer chamber by rotating the dish carefully. DO NOT SHAKE - care must be taken not to transfer material between the two chambers. Check that the outer chamber turns pink, i.e., is alkaline.

Prepare a blank using distilled water instead of boric acid extract, but otherwise as described above.

Prepare diffusion controls in triplicate, substituting 1 ml of 1 percent ammonium sulphate for the boric acid extract in the outer chamber, but otherwise treat as above.

Carefully transfer the dishes to an incubator at approximately 37° C for 45-60 minutes or leave them at ambient temperature for about 24 hours. It is essential that all dishes are treated identically.

Remove dishes from the incubator and titrate into the centre chamber using 0.05 M (0.1 N) standardized sulphuric acid in a 5.0 ml microburette.

Note the titre.

Calculation of results

Using the diffusion controls calculate the percentage diffusion X, from a knowledge of the amount of TVB placed in the dish and the amount found in the centre chamber.

1 ml 0.05 M sulphuric acid = 1.4 mg TVB

It is assumed that all samples will show identical percentage diffusions, and incomplete diffusion in the unknowns is corrected for by multiplying the result by 100.

Subtract any value obtained for the blank if this is more than one drop of 0.05 M sulphuric acid. A high blank value suggests faulty reagents or more probably poor techniques.

The amount of total volatile bases is normally expressed as their content of milligrammes of nitrogen (mg N) per 100 g of fish.

Thiobarbituric acid (TBA) value

Reagents

4N hydrochloric acid
TBA reagent: 0.2883 g TBA (AR grade) per 100 cm^3 90 percent glacial acetic acid
Keep the container wrapped in aluminium foil

Method

Duplicate 10 g muscle samples are macerated in 50 cm^3 distilled water for 2 minutes. The sample is washed into a three-parallel necked 500 cm^3 round bottomed flask using a further 47.5 cm^3 distilled water. To bring this solution to approximately pH 1.5, 2.5 cm^3 HCl are added. A blank is also prepared, omitting the sample. (If the sample is very fatty and rancid, antifoam is added, in which case, a blank of water, acid and antifoam is run.)

Three glass balls are added and the flask placed in position in the malonaldehyde distillation apparatus. This is heated with a Bunsen burner. A distillate of 50 cm^3 is collected in a volumetric flask in 10 minutes (this is the maximum time allowed) from when boiling commences, i.e., the first drop of distillate in the volumetric flask. The sample must not boil too vigorously; otherwise it will overflow into the distillation apparatus. Wash the apparatus well between samples. The distillates are stored at 0° C (it is possible to store overnight).

Five cm^3 distillate are pipetted into a test tube in duplicate. Five cm^3 freshly prepared TBA reagent are added. This solution is shaken and heated in boiling water for 35 minutes. Five cm^3 of the blank distillate are also prepared in duplicate. The test tubes are cooled in cold water for 10 minutes. The absorbance of the solutions are measured at 538 nm against the blank.

Calculation

For each muscle sample, there are four absorbance readings:

$$\text{TBA value} = \frac{\text{mean of all four absorbance readings} \times 7.8 \times 10}{\text{Wt sample}}$$

Units: mg malonaldehyde/kg muscle

FURTHER READING

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